INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 25, No. 5, p. 13-23, 2024
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Citrus aurantium bark, seeds, and leaves were used to synthesize and characterize silver nanoparticle and their antimicrobial activity was evaluated

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Article published on November 03, 2024

Key words: Silver nanoparticle, *Citrus aurantium*, Green synthesis, Plant extract, Bark, Leaves, Seed

Abstract

The green synthesis of silver nanoparticles has been proposed as an eco-friendly and cost-effective substitute for chemical and physical methods. The aim of this study was to synthesize and characterize silver nanoparticles using the peel extract of *Citrus aurantium* Bark, Leaf and Seed, and to determine the possible phytochemical constituents' presence in the plant extracts that might be responsible for the synthesis. *Citrus aurantium* Bark, Leaf and Seed extraction was followed by phytochemical studies of secondary metabolites, FTIR analysis confirmation of functional groups analysis. Silver nanoparticles were synthesized through bio-reduction of silver ions to silver nanoparticles using *Citrus aurantium* Bark, Leaf and Seed and characterized using UV-Vis spectroscopy (Bark, Leaf and Seed), SEM (Bark and Leaf), XRD (Bark, Leaf and Seed) and FTIR (Bark Leaf and Seed). The FTIR analysis of the extract revealed the presence of functional groups like hydroxyl, carboxyl, carbonyl, amine, and phenyl with similar functional groups. The synthesized silver nanoparticle (AgNP) has displayed the characteristics of a UV-Vis spectroscopy band peak from 400–420 nm. The XRD analysis also confirmed that the nanoparticles synthesized are crystalline in nature. Based on the findings of this study, it is understood that the variety of natural compounds that are present in plant extracts of *Citrus aurantium* Bark, Leaf and Seed can act as both reducing and stabilizing agents for the synthesis of silver nanoparticles. It is, therefore, concluded that *Citrus aurantium* Bark, Leaf and Seed extract can be potentially used for the large production of silver nanoparticles for several applications.

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Introduction

Due to their potential and potential applications in a variety of fields, including biomedicine, nanomedicine, agriculture, and biosensors, there has been an increase in interest in the synthesis of metallic nanoparticles such as zinc, silver, platinum, and gold in recent years (Tijjani Mustapha *et al.*, 2023; Pirtarighat *et al.*, 2019). Due to their high stability and low chemical reactivity compared to other metals, silver nanoparticles have been studied more than any other nanomaterial. Because of their unique and promising qualities, they are frequently employed as larvicidal, antibacterial, and anticancer agents (Tijjani Mustapha *et al.*, 2023; Mustapha *et al.*, 2022). Nonetheless, two distinct approaches are frequently used to synthesize them: the chemical and physical approaches. Typically, chemical or physical techniques such as micelle synthesis, sol process, chemical precipitation, hydrothermal method, pyrolysis, and chemical vapour deposition are used to create nanomaterials (Charusheela Ramteke *et al.*, 2013; Leela and Vivekantandan, 2008). Certain techniques are simple and allow for the regulation of crystallite size through the restoration of the reaction environment. However, there are still issues with the product's overall stability and getting monodisperse nanosize using these techniques (Kowshik *et al.*, 2002; Charusheela Ramteke *et al.*, 2013). Furthermore, it has been discovered that a large number of conventional techniques are capital-intensive and inefficient in their use of materials and energy (Klaus-Joerger *et al.*, 2001; Charusheela Ramteke *et al.*, 2013).

A green method has recently been proposed to replace the methods that harm the environment, such as chemical and physical ones. The biological method also referred to as the green synthesis technique or method makes use of bacteria, fungi, and plants. Using plant extracts from different plant parts, including the peel, stem, leaf, root, and fruit, several studies have reported the green synthesis of silver nanoparticles (Charusheela Ramteke *et al.*, 2013; Atharbi *et al.*, 2018; Ayodele *et al.*, 2020; Kokila *et al.*, 2015). A flowering plant in the Rutacea family is called *Citrus aurantium*. The terms "bitter orange" and "key lime" are frequently used to describe them (Khan Pathan *et al.*, 2012; Nur *et al.*, 2016). It is one of the most widely used citrus species in Malaysia, where it is mostly utilized in traditional medicine and food. Its 3–5 m tall, spiky stem is its main feature. The citrus plant is spherical in shape, with leaves that are 3–5 cm thick and 5–9 cm long (Nur *et al.*, 2016; Daigy, 2009; Mandal *et al.*, 2009). Traditionally, tulsi leaves have been used to treat a variety of infections. It has been stated that the antibacterial activity stems from the components of essential oils, primarily the eugenols. The goal of this study is to create silver nanoparticles using Tulsi leaf aqueous extract. Additionally, in an effort to maximize antimicrobial action, we try combining the natural antibacterial properties of Tulsi extract with silver metal (Raghunan *et al.*, 2011; Dubey *et al.*, 2010; Baret *et al.*, 2009).

Even though there have been a number of studies on silver nanoparticles, more thorough research is still needed on the environmentally friendly synthesis of silver nanoparticles utilizing plant extracts (Gardea-Torresdey *et al.*, 2003; Rafique *et al.*, 2017). To our knowledge, no research has been done on the use of *Citrus aurantium* Bark, Leaf and Seed extract in the plant-mediated production of silver nanoparticle. Thus, identifying and characterizing the function of metabolites in the creation of silver nanoparticles constitutes the novelty of the current work. Considering the aforementioned, the purpose of this work was to use *Citrus aurantium* Bark, Leaf and Seed extract to synthesize and characterize silver nanoparticle and to identify potential phytochemical constituents present in the plant extracts that could be involved in the synthesis of the silver nanoparticle.

Materials and methods

Preparation of plants extract

The different parts of plants like bark, leaves and seeds *of Citrus aurantium* were collected from home garden and the campus of Sri Paramakalyani Centre of Excellence in Environmental Science, Manonmaniam Sundaranar University, Alwarkurichi

and Tamilnadu. The method of Zina Albahadly *et al.* (2019) was used to prepare the like bark, leaves and seeds *of Citrus aurantium* extract. First, it was taken 20 grams of bark, leaves and seeds *of Citrus aurantium* and was washed repeatedly by distilled water to clean them by remove the dust and organic impurities which they present in it as shown in Fig. 1 and finely chopped and boiled it with 100 ml double distilled water at 60-80°C for 10 min. After that, the solution was filtered through nylon mesh cloth and stored at 4°C for further nanoparticle synthesis process. Finally, the solutions were filtered through filter papers many times.

Fig. 1. Tree of *Citrus aurantium* of bark, leaf and seed

Biosynthesis of silver nanoparticle

Sterile deionized triple - distilled water was used to synthesis a stock solution from $AgNO_3$, which were utilized in the next dilutions, later. AgNPs were made according to the method described by Gurunathan *et al.* (2013); Sujatha *et al.* (2013). Zina Albahadly *et al.* (2019). First, it was taken 20 ml of aqueous extract of bark, leaves and seeds *of Citrus aurantium* that were filled with sterile distilled water to a total 50 ml. After that, the solution is added to 5 ml of $AgNO_3$ solution and then exposed for 24hrs under room condition. The yellow color of mixture solution was turned to dark yellow, when the solution put in incubation for 24 hours, which are indicating that silver nanoparticles were formatted (Sujatha *et al.*, 2013; Zina Albahadly *et al.*, 2019) indicates the formation of silver nanoparticles and the absorbance of silver nanoparticles in the solution were monitored at different time interval using UV-vis spectroscopy.

Antibacterial activity of biosynthesized silver nanoparticles

The silver nanoparticles synthesized using *Citrus aurantium* extract of bark, leaves and seeds tested for antimicrobial activity by agar well diffusion method against *Bacillus cereus, Pseudomonas aeruginosa*. Luria Bertani Agar medium was used to cultivate bacteria. Fresh overnight culture of each strain was swabbed uniformly onto the individuals plates using sterile cotton swabs. 5 wells were made on each Luria Bertani Agar plates. Then the centrifuged silver nanoparticles (10, 20, 30, 40 and 50 µL) were poured into each well on all plates and incubate for 24 h at 37°C. After incubation the different levels of zonation formed around the well was measured.

Results and discussion

Ultraviolet–visible spectroscopy analysis (UV)

Reduction of silver ions into silver nanoparticles by using plant extracts like Bark Extract, Leaf extract and Seed Extract of *Citrus aurantium* was characterized by UV-vis spectra. Fig. 2-4 show the UV-vis spectra of reaction medium of silver nitrate with extracts of Bark Extract (Fig. 2), Leaf extract (Fig. 3) and Seed Extract (Fig. 4) of *Citrus aurantium* respectively were exposed with different time intervals. Fig. 2, 3 and 4 represents silver nanoparticle synthesis using Bark Extract, Leaf extract and Seed Extract of *Citrus aurantium* reaction time of 30 min, 1 h, 2 h, 3 hr, 4 h and 24 h. The maximum absorption band occurs at the wavelength of 460 nm due to the excitations of surface plasmon resonance in the nanoparticles and the broadening peak indicates particles are polydispersed. While adding the Bark Extract, Leaf

extract and Seed Extract of *Citrus aurantium* extract into the silver nitrate solution the reduction starts with in 10 min and steadily increases in absorbance as function of reaction time indicates that the continuous formation of silver nanoparticles. After 24 h of reaction time the absorbance was decreased indicates the completion of reaction. The optimum time required for completion of reaction was recorded as 24 h. Silver nanoparticle formation was started at 10 min is similar to the report of by Amin *et al.* (2012) where they used *Solanum xanthocarpum* berry extract.

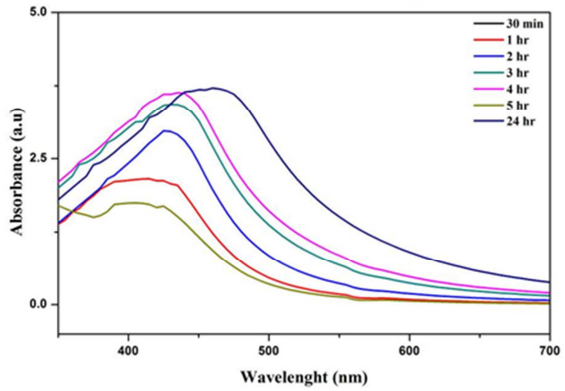

Fig. 2. UV- Vis spectra recorded the synthesis of silver nanoparticles. The silver nitrate 1mM was added to the Bark Extract of Citrus aurantium and incubated at different growth periods like 30min – 24 h

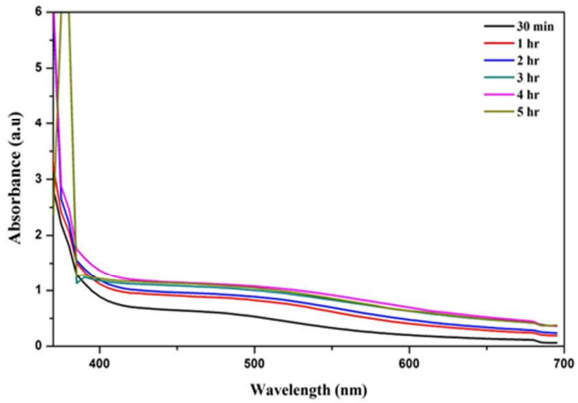

Fig. 3. UV- Vis spectra recorded the synthesis of silver nanoparticles. The silver nitrate 1mM was added to the leaf extract of *Citrus aurantium* and incubated at different growth periods like 30min – 24 h

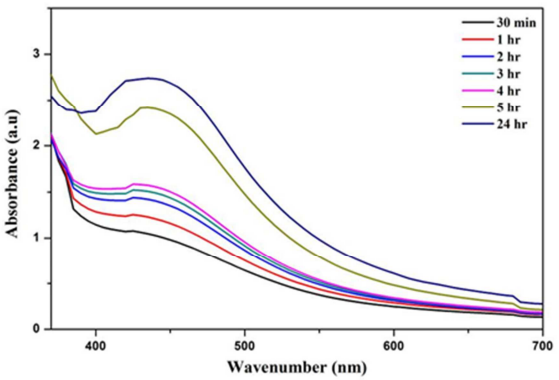

Fig. 4. UV- Vis spectra recorded the synthesis of silver nanoparticles. The silver nitrate 1mM was added to the seed extract of *Citrus aurantium* and incubated at different growth periods like 30min – 24 h

The absorbance is gradually increases at different functional time without any shift in the peak wavelength due to the excitation of longitudinal plasmon vibrations of silver nanoparticles in the solution (Kamat *et al.*, 1998). The reduction is started at 5 min and slowly increases up to 2 h, after 2 h the absorbance for silver nanoparticles was decreased. The absorbance intensity of 2 h reaction time located near to that at 3 h indicates completion of reaction. The broad band and low intensity results the formation of large sized nanoparticles. The reduction of silver ions into silver nanoparticles occurred rapidly within 2 h of reaction. Govindaraju *et al.* (2010) observed that the nanoparticles formation was completed within 60 min where they used the leaf extract of *Solanum torvum*. The narrow peak and increasing absorbance was observed from 5 min to 4 h without any shift of plasmon resonance band (Fig. 2-4). The absorbance was increased at the incubation time of 16 h and broad band was formed at 460 nm. The reaction is completed at 4 h is visually identified by appearance of precipitation in the bottom of the flask. Fig. 2-4 shows formation of nanoparticles using extract of Bark Extract, Leaf extract and Seed Extract of *Citrus aurantium* as a function of reaction time. When the time duration increased the nanoparticles synthesis also increased. Nanoparticles formation was initiated with in a min. Initially, the broad band was located at 460 nm due to the formation of larger

particles. After 3 h, the peak was slightly shift into 435 nm indicates size reduction of nanoparticles and it attained maximum at 24 h of incubation. At 24 h of incubation the broad band was slightly changed into narrow peak. There is no change in peak position suggesting that nucleation of silver nanoparticles starts with initiation of reaction time only and the size remains unchanged throughout the course of reaction. Previously, Kumar *et al.* (2012) reported the silver nanoparticles formation was completed in 4 h using the agricultural waste of *Annona squamosa* peel extract.

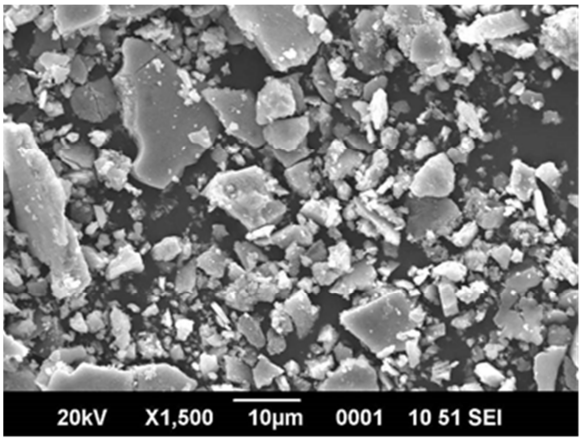

Fig. 5. SEM analyses of silver nanoparticles. SEM images shows some spherical-shaped and poly dispered silver nanoparticles synthesized using Bark Extract of *Citrus aurantium*

Scanning electron microscope (SEM)

SEM image (Fig. 5 and 6) of silver nanoparticles using a scanning electron microscope to characterize the size and shape of the nucleating aggregates through inclusion of a surface stabilizer (Deshpand *et al.*, 2010). The typical morphologies of such as triangle, pseudo triangle in Fig. 5 for bark extract mediated synthesis of silver nanoparticles and irregular spherical in shape in Fig. 6 was obtained by leaf extract synthesis of silver nanoparticles. The synthesized silver nanoparticles using *Citrus aurantium* bark and leaf are irregular in shape with sizes ranging between in the range of 32 to 45 nm, 95 to 125 nm respectively. The particles are well-dispersed with a more irregular size. The images suggest that the silver nanoparticles are synthesized due to the achievement of biomass, which perform as a silver bioreductant for biosynthesis. There are few traces of silver nanoparticles clusters which may possibly contribute for the difference in particle size.

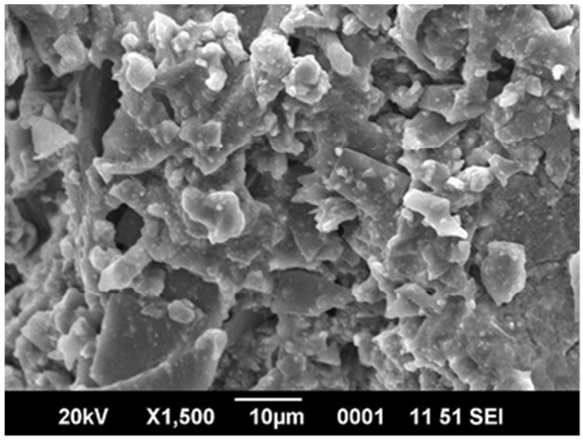

Fig. 6. SEM analyses of silver nanoparticles. SEM images shows some spherical-shaped and poly dispered silver nanoparticles synthesized using Leaf Extract of *Citrus aurantium*

X-Ray diffraction analysis (XRD)

Crystalline size and structure of the silver nanoparticles were determined by XRD in the whole spectrum of 2θ values ranging from 20-80. The green synthesis of silver nanostructure by employing extract of Bark Extract, Leaf extract and Seed Extract of *Citrus aurantium* was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Fig. 7-9). The four distinct diffraction peaks of the 2θ plane values of (111) (200) (222) and (311) for Bark Extract of *Citrus aurantium* (Fig. 7). The 2θ plane values of (111) (200) (222) and (311) for Leaf extract of *Citrus aurantium* (Fig. 8). The 2θ plane values of (111) (200) (222) and (311) for Seed Extract of *Citrus aurantium* (Fig. 9). Fig. 9 could be assigned the plane of (1 1 1), (2 0 0), (2 2 0) and (3 1 1), respectively, indicates the silver nanoparticles are fcc and crystalline in nature. The synthesized silver nanoparticles are compared with standard silver nitrate and pure silver particles which are published by Joint Committee on Powder Diffraction Standards (File nos. 04-0783 and 84-0713). A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals.

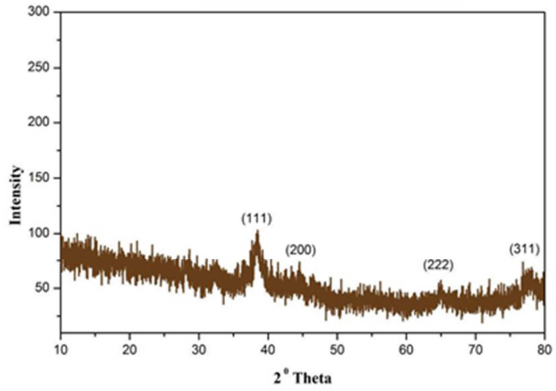

Fig. 7. XRD of the air-dried silver nanoparticles synthesized using bark extract of *Citrus aurantium*

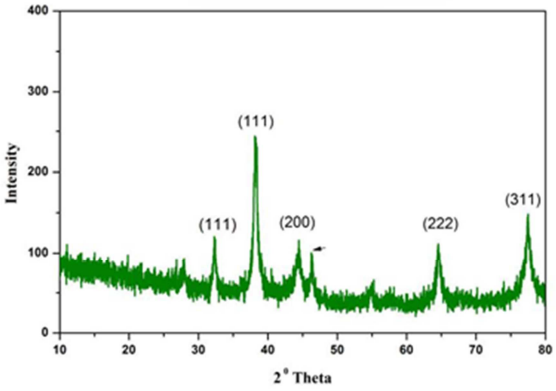

Fig. 8. XRD of the air-dried silver nanoparticles synthesized using leaf extract of *Citrus aurantium*

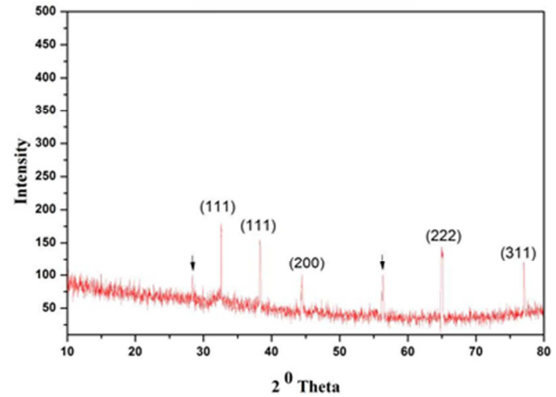

Fig. 9. XRD of the air-dried silver nanoparticles synthesized using seed extract of *Citrus aurantium*

A few unassigned peaks (marked with stars) were also observed that suggest the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Fig. 7-9). Similar results was obtained by using plant systems such as leaves (Shankar *et al.,* 2003), flowers and fruits (Dubey *et al.*, 2010), latex (Bar *et al.,* 2009a; 2009b), seed (Raghunandan *et al.*, 2011), peel (Kumar *et al.*, 2012) and mushroom extract (Daizy, 2009).

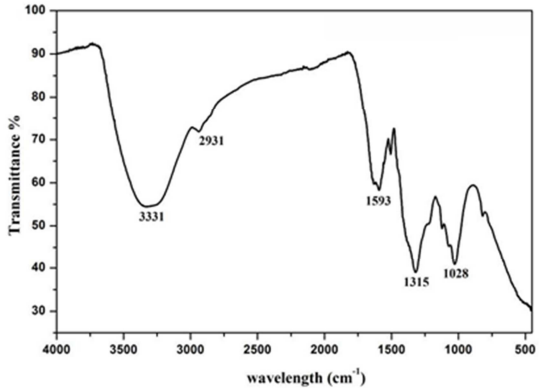

Fig. 10. FTIR Spectrum of silver nanoparticles using bark extract of *Citrus aurantium*

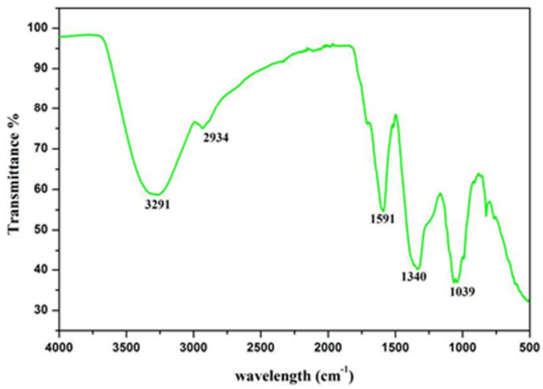

Fig. 11. FTIR Spectrum of silver nanoparticles using leaf extract of *Citrus aurantium*

Fourier transform infra-red spectrophotometer (FTIR)

FT-IR shows (Fig. 10-12) the functional molecules of Bark Extract, Leaf extract and Seed Extract of *Citrus aurantium* associated with silver nanoparticles, which are involved in the reduction of silver ions into nanoparticles. The peak at 3314 cm^{-1} shows O-H stretching of carboxylic acid, the small peak at 2931

cm^{-1} due to the C-H stretching vibrations of aromatic grop, peak at 1593 cm^{-1} corresponding to C-H stretching (in ring) to aromatic groups. The strong and narrow peak at 1315 cm^{-1} are most probably on account of N-O functional group, the narrow and strong peak at 1028 cm^{-1} indicates presence of C–N stretch aliphatic amines (Fig. 10).

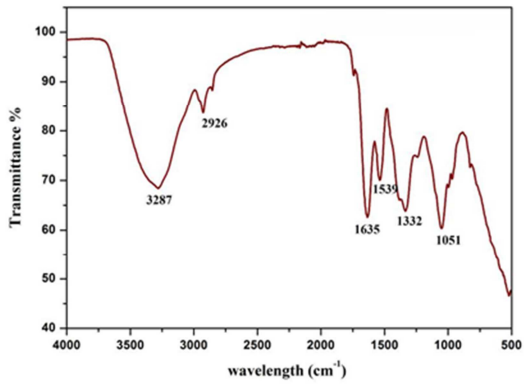

Fig. 12. FTIR Spectrum of silver nanoparticles using seed extract of *Citrus aurantium*

Table 1. FTIR analysis and functional groups of extract of bark and seed of *Citrus aurantium*

Citrus aurantium extracts	Wavenumber (cm^{-1})	Functional groups
Bark extracts	3331 cm^{-1}	O-H Stretch
	2931 cm^{-1}	C-H Stretch
	1593 cm^{-1}	C-C Stretch (in ring)
	1315 cm^{-1}	N-O Symmetric stretch
	1028 cm^{-1}	C-N Stretch
Leaf extract	3291 cm^{-1}	-C≡C-H:O-H Stretch
	2934 cm^{-1}	C-H Stretch
	1591 cm^{-1}	C-H Stretch (in ring)
	1340 cm^{-1}	N-O Symmetric Stretch
	1039 cm^{-1}	C-N Stretch
Seed extracts	3287 cm^{-1}	-C≡C-H:O-H Stretch
	2926 cm^{-1}	C-H Stretch
	1635 cm^{-1}	-C≡H Stretch
	1539 cm^{-1}	N-O Asymmetric Stretch
	1332 cm^{-1}	N-O Symmetric Stretch
	1051 cm^{-1}	C-N Stretch

In Fig. 11 shows the 3287 cm^{-1}, 2926 cm^{-1}, 1635 cm^{-1}, 1539 cm^{-1}, 1332 cm^{-1} and 1051 cm^{-1} corresponding to -C≡C-H:O-H Stretching, C-H Stretching, -C≡H Stretching, N-O Asymmetric Stretching, N-O Symmetric Stretching and C-N Stretching indicates presence of alkens (terminal), aromatic, alkens, nitro componds and aliphatic amino groups. In an earlier study, Kumar *et al.* (2007) used the enzyme nitrate reductase purified from *Fusarium oxysporum* and a peptide, phytochelatin, for the in vitro synthesis of silver nanoparticles in the presence of a co-factor, α-NADPH shown in Table 1.

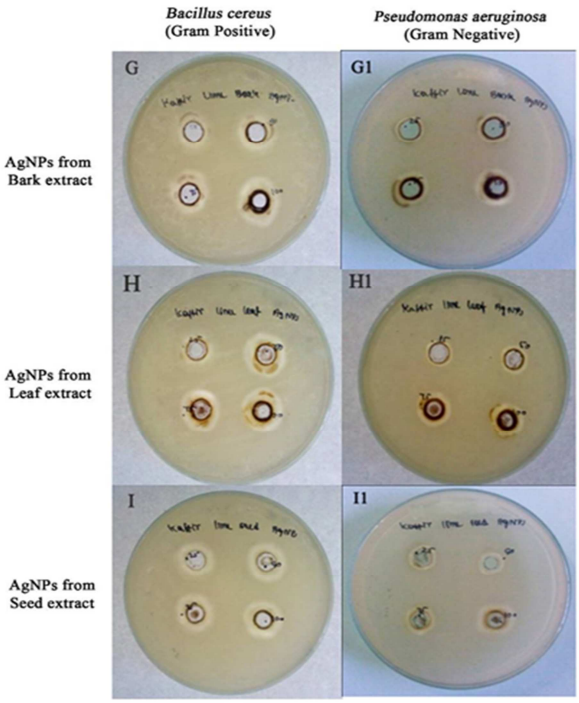

Fig. 13. Antibacterial activity of AgNPs synthesized from Bark Extract, Leaf extract and Seed Extract of *Citrus aurantium*

Silver nanoparticles antimicrobial activity

The well diffusion method was carried out to assay of antibacterial activity of green approach silver nanoparticles under in vitro conditions against Bark Extract, Leaf extract and Seed Extract of *Citrus aurantium* with different antibacterial agents involved silver as an antibacterial agent possesses great potential and can be used efficiently in many diverse applications (Sharma *et al.*, 2009). Silver nanoparticles are an eminent antiviral, antifungal and antibacterial agent. Silver nanoparticles always have unique physical and chemical properties due to their size, shape, dispersity and surface area, which provide better contact with microorganisms so shown to have immense impact on the antibacterial efficacy (Rai *et al.*, 2009; Govindaraju *et al.*, 2010; Ray and Bark, 2010). The silver nanoparticles (bark, leaf and seed

extracts of *Citrus aurantium* exhibits admirable zone of inhibition at 100 µl against *Bacillus cereus* and *Pseudomonas aeruginosa* as shown in Fig. 14, 15 and 16. Similarly, the silver nanoparticles (bark, leaf and seed extracts of, *Citrus aurantium* show the maximum zone of inhibition of against *Bacillus Subtilis* and *Enterococcus* respectively as shown in Table 2.

Table 2. Antibacterial activity of AgNPs synthesized from bark extract, leaf extract and seed extract of *Citrus aurantium*

AgNPs from nutmeg plant extract	test organisms	Zone of inhibition (mm)			
		25 µl	50 µl	75 µl	100 µl
AgNPs from bark extract	*Bacillus cereus*	13.33 ± 0.333	14 ± 0.577	14.5 ± 0.5	15 ± 0.577
	Pseudomonas aeruginosa	5.666 ± 0.333	6.333 ± 0.333	6.833 ± 0.440	7.666 ± 0.333
AgNPs from leaf extract	*Bacillus cereus*	11.666 ± 0.333	12.666 ± 0.333	13.666 ± 0.333	14.333 ± 0.333
	Pseudomonas aeruginosa	5.333 ± 0.333	6 ± 0.288	6.333 ± 0.333	7 ± 0.577
AgNPs from seed extract	*Bacillus cereus*	11.25 ± 0.478	11.666 ± 0.666	12.666 ± 0.666	13.666 ± 0.666
	Pseudomonas aeruginosa	5.666 ± 0.333	6.166 ± 0.166	6.833 ± 0.166	7.333 ± 0.333

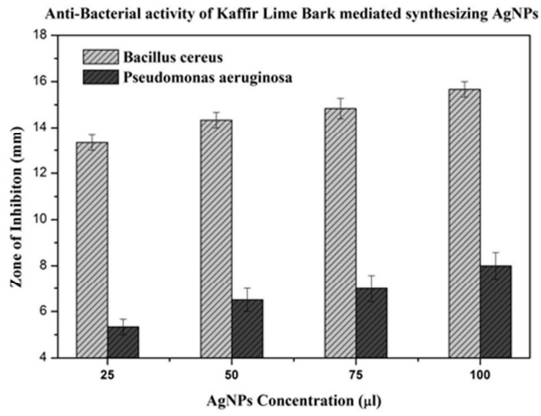

Fig. 14. Antibacterial activity of AgNPs synthesized from bark extract of *Citrus aurantium*

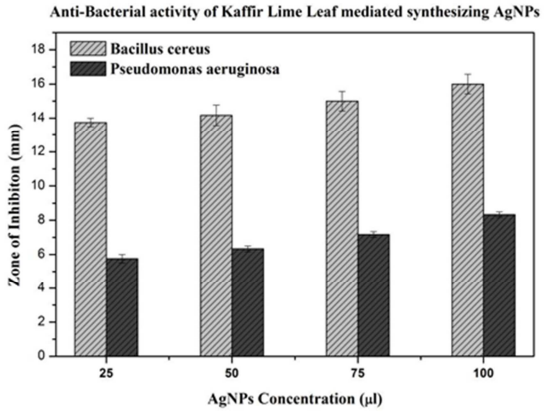

Fig. 15. Antibacterial activity of AgNPs synthesized from leaf extract of *Citrus aurantium*

The concentration of silver nanoparticles was varied from 25 ul, 50 ul, 75 ul and 100 ul. The inhibition of zone increased while increasing concentration of silver nanoparticles. The zone of inhibition in diameter was tabulated shows in Table. 2. The table showed an inhibition zone at the various concentrations of silver nanoparticles synthesized from plants extract of *Citrus aurantium* bark, leaf and seed. Fig. 14 shows the zone of inhibition of *Bacillus cereus* and *Pseudomonas aeruginosa* with the different concentrations of silver nanoparticles from nutmeg bark water extract (Fig. 13G and G1), representing to the Table 2, 13.33±0.333, 14±0.577, 14.5±0.5, 15±0.577 and 5.666±0.333, 6.333±0.333, 6.833±0.44, 7.666±0.333.

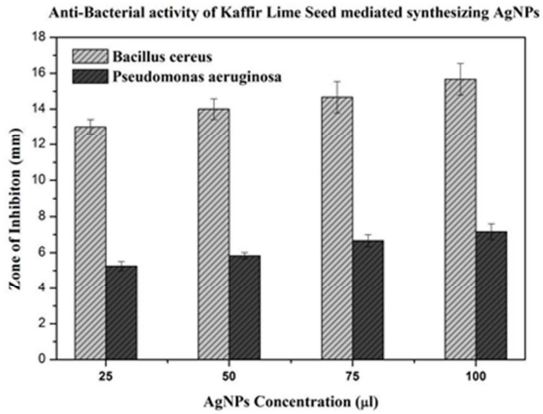

Fig. 16. Antibacterial activity of AgNPs synthesized from seed extract of *Citrus aurantium*

Fig. 13 H and H1 show the zone of inhibition with different concentration of plants extract of *Citrus aurantium* bark, leaf and seed mediated synthesized silver nanoparticles. Comparison of two different

species bacterial inhibition shows in Fig. 14 with the corresponding inhibition values are representing in the Table 2 with the values of 11.666±0.333, 12.666±0.333, 13.666±0.333, 14.333±0.333 and 5.333±0.333, 6±0.288, 6.333±0.333, 7±0.577. *Citrus aurantium* Extract of Bark Extract, Leaf extract and Seed mediated synthesized silver nanoparticle inhibit the growth of above two different bacterial species was shows in the Fig. 13 I and I1 with the inhibition values of 11.25±0.478, 11.666±0.666, 12.666±0.666, 13.666±0.666 and 5.666±0.333, 6.166±0.166, 6.833±0.166, 7.333±0.333 represented by Table 2. The comparison inhibition of two different species of bacteria (*Bacillus cereus* and *Pseudomonas aeruginosa*) with different concentration of plants extract of *Citrus aurantium* bark, leaf and seed mediated synthesize silver nanoparticle are show in the Fig. 16.

Conclusion

The field of nanotechnology is the most active area of research in modern materials science. Though there are many chemical as well as physical methods, green synthesis of nanomaterials is the most emerging method of synthesis. In the current study, silver nanoparticles were successfully fabricated via the reduction of silver ions (Ag^+) by the secondary metabolites present in *Citrus aurantium* bark, leaf and seed extract. Different types of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, phenols, and steroid were identified in the *Citrus aurantium* bark, leaf and seed extract. Hence, *Citrus aurantium* fruit peel extract can act as a good candidate for the reduction of silver ions to silver nanoparticles. It is concluded that the green synthesis approach appears to be a cost-effective alternative to the conventional physical and chemical methods. Moreover, the *Citrus aurantium* can be potentially used for the large production of silver nanoparticles for various applications. The present study contributes to the understanding of green synthesis of silver nanoparticles using *Citrus aurantium* bark, leaf and seed extract. We report the synthesis of antibacterial silver nanoparticles using leaf broth of medicinal herb, *Citrus aurantium*. The synthesized AgNPs have been characterized by UV-Vis spectroscopy, FTIR, SEM, X-ray diffractometry. Such AgNPs stabilized by *Citrus aurantium* Bark, Seed and leaf extract were found to have enhanced antimicrobial activity against well-known pathogenic strains, namely *Bacillus cereus, Pseudomonas aeruginosa* reveals high potential of *Citrus aurantium* extract stabilized AgNPs to be used as antimicrobial agent in medical field as well as food and cosmetic industries.

Acknowledgements

Venkateshwari R (Register No: 22214012052022) acknowledges Sri Paramakalyani centre of Excellence in Environmental Science, Manonmaniam Sundaranar University, Alwarkurichi, India, Providing the support for this research work. The author Venkateshwari R. – IF 220080 would like to gratefully acknowledge the Department of Science and Technology for providing research funding under the Inspire Fellowship scheme grant number DST/INSPIRE FELLOWSHIP/2022/IF220080.

References

Albahadly Z, Albahrani R, Hamza A. 2019. Silver nanoparticles synthesized from *Citrus aurantium* L. & *Citrus sinensis* L. leaves and evaluation of antimicrobial activity. Journal of Global Pharma Technology **11**(3), 71-75.

Amin M, Anwar F, Janjua MRSA, Iqbal MA, Rashid U. 2012. Green synthesis of silver nanoparticles through reduction with *Solanum xanthocarpum* L. berry extract: Characterization, antimicrobial and urease inhibitory activities against *Helicobacter pylori*. Int. J. Mol. Sci **13**, 9923-9941.

Ayodele M, Chikodiri V, Adebayo-Tayo BC. 2020. Green synthesis and cream formulations of silver nanoparticles of *Nauclea latifolia* (African peach) fruit extracts and evaluation of antimicrobial and antioxidant activities. Sustain. Chem. Pharm **15**, 100197.

Bar H, Bhui DH, Sahoo PG, Sarkar P, De PS, Misra A. 2009a. Green synthesis of silver nanoparticles using latex of *Jatrapha curcas*. Colloids Surf A Physicochem Eng Asp **339**, 134–139.

Bar H, Bhui DK, Sahoo GP, Sarkar P, Pyne S, Misra A. 2009b. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids Surf A Physicochem Eng Asp **348**, 212–216.

Charusheela R, Tapan C, Bijaya KS, Ram-Avatar P. 2013. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. Journal of Chemistry **278925**, 1-7.

Daizy P. 2009. Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract. Spectrochimica Acta Part A **73**, 374–381.

Deshpande R, Bedre MD, Basavaraja S, Sawle B, Manjunath SY, Venkataraman A. 2010. Rapid biosynthesis of irregular shaped gold nanoparticles from macerated aqueous extracellular dried clove buds (*Syzygium aromaticum*) solution. Colloids and Surfaces B: Biointerfaces **79**, 235–240.

Dubey SP, Lahtinen M, Sillanpaa M. 2010. Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of *Rosa rugosa*. Colloids and Surfaces A: Physicochem. Eng. Aspects **364**, 34–41.

Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose-Yacaman M. 2003. Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles. Langmuir **19**, 1357–1361.

Govindaraju K, Basha SK, Ganesh Kumar V, Singaravelu G. 2008. Silver, gold and bimetallic nanoparticles production using single-cell protein (*Spirulina platensis* Geitler). J Mater Sci **43**, 5115–5122.

Govindaraju K, Tamilselvan S, Kiruthiga V, Singaravelu G. 2010. Biogenic silver nanoparticles by *Solanum torvum* and their promising antimicrobial activity. Journal of Biopesticides **3**(1), 394–399.

Gurunathan S, Raman J, AbdMalek SN, John PA, Vikineswary S. 2013. Green synthesis of silver nanoparticles using *Ganoderma neojaponicum* Imazeki: a potential cytotoxic agent against breast cancer cells. Int. J. Nanomedicine **8**, 4399-4413.

Kamat PV, Flumiani M, Hartland GV. 1998. Picosecond dynamics of silver nanoclusters: photo ejection of electrons and fragmentation. J. Phys. Chem. B **102**, 3123–3128.

Khan Pathan R, Gali PR, Pathan P, Gowtham T, Pasupuleti S. 2012. In vitro antimicrobial activity of *Citrus aurantifolia* and its phytochemical screening. Asian Pac. J. Trop. Dis **2**, S328–S331.

Klaus-Joerger T, Joerger R, Olsson E, Granqvist CG. 2001. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. Trends in Biotechnology **19**(1), 15–20.

Kokila T, Ramesh PS, Geetha D. 2015. A biogenic approach for green synthesis of silver nanoparticles using peel extract of *Citrus sinensis* and its application. Int. J. Chem. Tech. Res **7**(2), 804-813.

Kowshik M, Deshmukh N, Vogel W, Urban J, Kulkarni SK, Paknikar KM. 2002. Microbial synthesis of semiconductor CdS nanoparticles, their characterization, and their use in the fabrication of an ideal diode. Biotechnology and Bioengineering **78**(5), 583–588.

Kumar KP, Paul W, Sharma CP. 2012. Green synthesis of silver nanoparticles with *Zingiber officinale* extract and study of its blood compatibility. BioNanoSci **2**, 144–152.

Kumar R, Roopan SM, Prabhakarn A, Khanna VG, Chakroborty S. 2012. Agricultural waste *Annona squamosa* peel extract: biosynthesis of silver nanoparticles. Spectrochim. Acta Part A **90**, 173–176.

Leela A, Vivekanandan M. 2008. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. African Journal of Biotechnology **7**(17), 3162–3165.

Mondal SBR, Mirdha S, Mahapatra C. 2009. The science behind sacredness of Tulsi (*Ocimum sanctum* Linn.). Indian Journal of Physiology and Pharmacology **53**(4), 291–306.

Mustapha T, Ithnin NR, Othman H, Abu Hasan ZI, Misni N. 2023. Bio-fabrication of silver nanoparticles using *Citrus aurantifolia* fruit peel extract (CAFPE) and the role of plant extract in the synthesis. Plants **12**(8), 1648.

Mustapha T, Misni N, Ithnin NR, Daskum AM, Unyah NZ. 2022. A review on plants and microorganisms mediated synthesis of silver nanoparticles, role of plant metabolites and applications. Int. J. Environ. Res. Public Health **19**, 674.

Nur S, Othman A, Hassan MA, Nahar L, Basar N, Jamil S, Sarker SD. 2016. Essential oils from the Malaysian *Citrus* (*Rutaceae*) medicinal plants. Medicines **3**, 13.

Pirtarighat S, Ghannadnia M, Baghshahi S. 2019. Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment. J. Nanostructure Chem **9**, 1–9.

Rafique M, Sadaf I, Rafique MS, Tahir MB. 2017. A review on green synthesis of silver nanoparticles and their applications. Artif. Cells Nanomed. Biotechnol **45**, 1272–1291.

Raghunandan D, Borgaonkar PA, Bendegumble B, Bedre MD, Bhagawanraju M, Yalagatti MS, Huh DS, Abbaraju V. 2011. Microwave-assisted rapid extracellular biosynthesis of silver nanoparticles using carom seed (*Trachyspermum copticum*) extract and in vitro studies. American Journal of Analytical Chemistry **2**, 475-483.

Rai M, Yadav A, Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv **27**, 76–83.

Roy N, Barik A. 2010. Green synthesis of silver nanoparticles from the unexploited weed resources. International Journal of Nanotechnology **4**, 95.

Sharma VK, Yingard RA, Lin Y. 2009. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv in Colloid and Interf Sci **145**, 83-96.

Sujatha S, Tamilselvi Subha K, Panneerselvam1 A. 2013. Studies on biosynthesis of silver nanoparticles using mushroom and its antibacterial activities. Int. J. Curr. Microbiol. App. Sci **2**(12), 605-614.